AMENDMENTS TO THE CLAIMS

Listing of Claims:

1. (Currently amended) A method for producing a transgenic plant comprising:

i) transforming a plant cell with a first expression cassette comprising a nucleic acid sequence encoding a D-amino acid oxidase operably linked with a promoter allowing expression in plant cells or plants, in combination with at least one second expression cassette suitable for conferring to said plant an agronomically valuable trait, and

ii) providing at least one first compound X, which is phytotoxic against plant cells not functionally expressing said D-amino acid oxidase, wherein said compound X can be metabolized by said D-amino acid oxidase into one or more compound(s) Y which are non-phytotoxic or less phytotoxic than compound X, and

iii) treating said transformed plant cells of step i) with said first compound X in a phytotoxic concentration and selecting plant cells comprising in their genome both said first and said second expression cassette, wherein said first expression cassette is conferring resistance to said transformed plant cells against said compound X by expression of said D-amino acid oxidase, and

iv) providing at least one second compound M, which is non-phytotoxic or moderately phytotoxic against plant cells not functionally expressing said D-amino acid oxidase, wherein said compound M can be metabolized by said D-amino acid oxidase into one or more compound(s) N which are phytotoxic or more phytotoxic than compound M, and

v) breaking the combination between said first expression cassette and said second expression cassette and treating resulting said plant cells with said second compound M in a concentration toxic to plant cells still comprising said first expression cassette, and selecting plant cells comprising said second expression cassette but lacking said first expression cassette,

wherein said first compound X and said second compound M both comprise a D-amino acid structure.

2. (Original) The method of Claim 1, wherein said first expression cassette for said D-amino acid oxidase and said second expression cassette for said agronomically valuable trait are

a) both comprised in one DNA construct and combination is broken by deletion or excision of said first expression cassette for said D-amino acid oxidase, or

b) are comprised on separate DNA constructs which are transformed in combination by co-transformation into said plant cells, and combination is broken by subsequent segregation of the two expression cassettes.

3. (Previously presented) The method of Claim 1, wherein said method for producing a transgenic plant comprises the steps of:

i) transforming a plant cell with a first DNA construct comprising

a) a first expression cassette comprising a nucleic acid sequence encoding a D-amino acid oxidase operably linked with a promoter allowing expression in plant cells or plants, wherein said first expression cassette is flanked by sequences which allow for specific deletion of said first expression cassette, and

b) at least one second expression cassette suitable for conferring to said plant an agronomically valuable trait, wherein said second expression cassette is not localized between said sequences which allow for specific deletion of said first expression cassette, and

ii) providing at least one first compound X, which is phytotoxic against plant cells not functionally expressing said D-amino acid oxidase, wherein said compound X can be metabolized by said D-amino acid oxidase into one or more compound(s) Y which are non-phytotoxic or less phytotoxic than compound X, and

iii) treating said transformed plant cells of step i) with said first compound X in a phytotoxic concentration and selecting plant cells comprising in their genome said first DNA construct, conferring resistance to said transformed plant cells against said compound X by expression of said D-amino acid oxidase, and

iv) providing at least one second compound M, which is non-phytotoxic or moderately phytotoxic against plant cells not functionally expressing said D-amino acid oxidase, wherein said compound M can be metabolized by said D-amino acid oxidase into one or more compound(s) N which are phytotoxic or more phytotoxic than compound M, and

v) inducing deletion of said first expression cassette from the genome of said transformed plant cells and treating said plant cells with said second compound M in a concentration toxic to plant cells still comprising said first expression cassette, thereby selecting plant cells comprising said second expression cassette but lacking said first expression cassette.

4. (Previously presented) The method of claim 1 further comprising the step of regeneration of a fertile plant.

5. (Currently amended) The method of claim 1, wherein said first compound X comprises a D-amino acid structure selected from the group consisting of D-tryptophane, D-histidine, D-arginine, D-threonine, D-methionine, D-serine, and D-alanine~~, or derivatives thereof~~.

6. (Currently amended) The method of claim 1, wherein said second compound M comprises a D-amino acid structure selected from the group consisting of D-isoleucine, D-valine, D-asparagine, D-leucine, D-lysine, D-proline, and D-glutamine~~, or derivatives thereof~~.

7. (Previously presented) The method of claim 1, wherein deletion of said first expression cassette for the D-amino acid oxidase is realized by a method selected from:

a) recombination induced by a sequence specific recombinase, wherein said first expression cassette is flanked by corresponding recombination sites in a way that recombination between said flanking recombination sites results in deletion of the sequences in-between from the genome, or

b) homologous recombination between homology sequences A and A' flanking said first expression cassette, induced by a sequence-specific double-strand break caused by a sequence specific endonuclease, wherein said homology sequences A and A' have sufficient length and homology in order to ensure homologous recombination between A and A', and having an orientation which – upon

recombination between A and A' – will lead to excision of said first expression cassette from the genome of said plant.

8. (Previously presented) The method of Claim 7, wherein the recombinase or sequence-specific endonuclease, respectively, is expressed or combined with its corresponding recombination or recognition site, respectively, by a method selected from the group consisting of:

a) incorporation of a second expression cassette for expression of the recombinase or sequence-specific endonuclease operably linked to a plant promoter into said DNA construct, together with said first expression cassette flanked by said sequences which allow for specific deletion,

b) incorporation of a second expression cassette for expression of the recombinase or sequence-specific endonuclease operably linked to a plant promoter into the plant cells or plants used as target material for the transformation thereby generating master cell lines or cells,

c) incorporation of a second expression cassette for expression of the recombinase or sequence-specific endonuclease operably linked to a plant promoter into a separate DNA construct, which is transformed by way of co-transformation with said first DNA construct into said plant cells, and

d) incorporation of a second expression cassette for expression of the recombinase or sequence-specific endonuclease operably linked to a plant promoter into the plant cells or plants which are subsequently crossed with plants comprising the DNA construct of the invention.

9. (Previously presented) The method of Claim 7, wherein deletion of said first expression cassette for the D-amino acid oxidase is induced or activated by inducing expression and/or activity of said sequence-specific recombinase or endonuclease by a method selected from the group consisting of

a) inducible expression by operably linking the sequence encoding said recombinase or endonuclease to an inducible promoter, and

b) inducible activation, by employing a modified recombinase or endonuclease comprising a ligand-binding-domain, wherein activity of said modified recombinase or endonuclease can by modified by treatment of a compound having binding activity to said ligand-binding-domain.

10. (Previously presented) The method of claim 2, wherein the DNA construct comprises

a) a first expression cassette comprising a nucleic acid sequence encoding a D-amino acid oxidase operably linked with a promoter allowing expression in plant cells or plants, wherein said first expression cassette is flanked by sequences which allow for specific deletion of said first expression cassette, and

b) at least one second expression cassette suitable for conferring to said plant an agronomically valuable trait, wherein said second expression cassette is not localized between said sequences which allow for specific deletion of said first expression cassette

and the resulting plant cell or plant is selection marker free.

11. (Previously presented) A DNA construct suitable for the method of claim 1, comprising

a) a first expression cassette comprising a nucleic acid sequence encoding a D-amino acid oxidase operably linked with a promoter allowing expression in plant cells or plants, wherein said first expression cassette is flanked by sequences which allow for specific deletion of said first expression cassette, and

b) at least one second expression cassette suitable for conferring to said plant an agronomically valuable trait, wherein said second expression cassette is not localized between said sequences which allow for specific deletion of said first expression cassette.

12. (Previously presented) The DNA construct of Claim 11, wherein said D-amino acid oxidase expressed from said first expression cassette has metabolizing activity against at least one D-amino acid and comprises the following consensus sequence:

[LIVM]-[LIVM]-H*-[NHA]-Y-G-x-[GSA]-[GSA]-x-G-x_5-G-x-A

wherein amino acid residues given in brackets represent alternative residues for the respective position, x represents any amino acid residue, and indices numbers indicate the respective number of consecutive amino acid residues.

13. (Currently amended) The DNA construct of Claim 11, wherein said D-amino acid oxidase has enzymatic activity against at least one of the amino acids selected from the group consisting of D-alanine, D-serine, D-isoleucine, D-valine~~, and derivatives thereof~~.

14. (Previously presented) The DNA construct of claim 11 wherein said D-amino acid oxidase is described by a sequence of the group consisting of sequences described by GenBank or SwisProt Acc.No. JX0152, O01739, O33145, O35078, O45307, P00371, P14920, P18894, P22942, P24552, P31228, P80324, Q19564, Q28382, Q7PWX4, Q7PWY8, Q7Q7G4, Q7SFW4, Q7Z312, Q82MI8, Q86JV2, Q8N552, Q8P4M9, Q8PG95, Q8R2R2, Q8SZN5, Q8VCW7, Q921M5, Q922Z0, Q95XG9, Q99042, Q99489, Q9C1L2, Q9JXF8, Q9V5P1, Q9VM80, Q9X7P6, Q9Y7N4, Q9Z1M5, Q9Z302, and U60066.

15. (Previously presented) The DNA construct of claim 11 wherein said D-amino acid oxidase is selected from the group of amino acid sequences consisting of

a) sequences described by SEQ ID NO: 2, 4, 6, 8, 10, 12, and 14,

b) sequences having a sequence homology of at least 40% with a sequence as described by SEQ ID NO: 2, 4, 6, 8, 10, 12, and 14, and

c) sequences hybridizing under low or high stringency conditions with a sequence as described by SEQ ID NO: 2, 4, 6, 8, 10, 12, and 14.

16. (Previously presented) The DNA construct of claim 11, wherein said sequences which allow for specific deletion of said first expression cassette are selected from the group of sequences consisting of

a) recombination sites for a sequences-specific recombinase arranged in a way that recombination between said flanking recombination sites results in deletion of the sequences in-between from the genome, and

b) homology sequences A and A' having a sufficient length and homology in order to ensure homologous recombination between A and A', and having an

orientation which – upon recombination between A and A' – will result in deletion of the sequences in-between from the genome.

17. (Previously presented) The DNA construct of Claim 16, wherein said recombination sites correspond to a recombinase selected from the group consisting of a cre recombinase, a FLP recombinase, a Gin recombinase, a Pin recombinase, and a R recombinase.

18. (Original) The DNA construct of Claim 16, wherein said DNA construct comprises a recognition site of at least 10 base pairs for a sequence specific endonuclease between said homology sequences A and A'.

19. (Previously presented) The DNA construct of Claim 18, wherein said recognition site corresponds to a sequence-specific endonuclease selected from the group consisting of homing endonucleases I-SceI, I-CpaI, I-CpaII, I-CreI, and I-ChuI and chimeras thereof with ligand-binding domains.

20. (Previously presented) The DNA construct of claim 16, wherein said DNA construct further comprises a expression cassette for the sequence specific endonuclease or recombinase suitable for mediating deletion of the first expression cassette for the D-amino acid oxidase.

21. (Previously presented) The DNA construct of Claim 20, wherein expression and/or activity of said sequence-specific recombinase or endonuclease can be induced and/or activated by a method selected from the group consisting of

a) inducible expression by operably linking the sequence encoding said recombinase or endonuclease to an inducible promoter, and

b) inducible activation, by employing a modified recombinase or endonuclease comprising a ligand-binding-domain, wherein activity of said modified recombinase or endonuclease can by modified by treatment of a compound having binding activity to said ligand-binding-domain.

22. (Previously presented) A transgenic vector comprising the DNA construct of claim 11.

23. (Previously presented) A transgenic cell comprising the DNA construct of claim 11 or a vector comprising said construct.

24. (Previously presented) The transgenic cell of Claim 23, wherein said cell is a plant cell.

25. (Previously presented) A transgenic, non-human organism comprising the DNA construct of claim 11, a vector comprising said construct, or a transgenic cell comprising said construct or vector.

26. (Previously presented) The transgenic, non-human organism of Claim 25 wherein said organism is a plant.

27. (Previously presented) The method of claim 3 further comprising the step of regeneration of a fertile plant.

28. (Currently amended) The method of claim 3, wherein said first compound X comprises a D-amino acid structure selected from the group consisting of D-tryptophane, D-histidine, D-arginine, D-threonine, D-methionine, D-serine, and D-alanine~~, or derivatives thereof~~.

29. (Currently amended) The method of claim 3, wherein said second compound M comprises a D-amino acid structure selected from the group consisting of D-isoleucine, D-valine, D-asparagine, D-leucine, D-lysine, D-proline, and D-glutamine~~, or derivatives thereof~~.